

## Claims

What is claimed is:

1. A method of polynucleotide synthesis, comprising:  
combining in a polymerization reaction mixture a thermostable  
polymerase, a template nucleic acid molecule, appropriate primers for the template  
nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic  
5 acid polyanion, wherein the temperature of the polymerization reaction mixture is at a  
temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase  
activity; and  
heating the polymerization reaction mixture to a temperature at which the  
nonnucleic acid polyanion dissociates from the thermostable polymerase, allowing the  
10 thermostable polymerase to recognize and provide polynucleotide synthesis on a  
primer annealed nucleic acid molecule.
2. The method of claim 1 wherein the polynucleotide synthesis is  
polymerase chain reaction
3. The method of claim 1 wherein the non-nucleic acid polyanion has a  
15 molecular weight of from 1500 to 500,000.
4. The method of claim 1 wherein the non-nucleic acid polyanion has a  
molecular weight of from 4,000 to 15,000.
5. The method of claim 1 wherein the non-nucleic acid polyanion has a  
molecular weight of from 5,000 to 10,000.
- 20 6. The method of claim 1 wherein the non-nucleic-acid polyanion is a  
synthetic organic polysulfate selected from the group poly(anetholsulfonic acid)  
polyvinyl sulfate and polystyrene sulfate.
7. The method of claim 6 wherein the non-nucleic acid polyanion is a  
sulfated oligo-or polysaccharide.
8. A method of polynucleotide synthesis, comprising:  
combining in a polymerization reaction mixture a thermostable polymerase, a  
template nucleic acid molecule, appropriate primers for the template nucleic acid  
molecule, at least one deoxynucleoside triphosphate, and a polymer or copolymer of  
5 sugars selected from the group consisting of glucose, N-acetyl-glucosamine,  
galactouronic acid, hyalouronic acid, Nacetyl-galactosamine and sulfated fucose,



wherein the temperature of the polymerization reaction mixture is at a temperature at which the polymer or copolymer inhibits thermostable polymerase activity;

heating the polymerization mixture to a temperature at which the template  
10 nucleic acid molecule is denatured from a double-stranded molecule to a single-stranded molecule;

cooling the polymerization mixture to a temperature of from about 45°C to about 65°C to allow appropriate primers to anneal to the single-stranded molecule; and

15 modifying the polymerization mixture to a temperature at which the polymer or copolymer is substantially dissociated from the thermostable polymerase and the thermostable polymerase recognizes and provides polynucleotide synthesis on primer annealed nucleic acid molecule.

9. The method of claim 8 wherein the sulfated polymer or copolymer of  
20 sugars is selected from the group consisting of dextran sulfate, fucoidan, heparin; heparan sulfate, chondroitin polysulfate, keratan polysulfate, xylar poly, sulfate, and pentosan polysulfate.

10. The method of claim 1 wherein the non-nucleic acid polyanion is at a final reaction concentration of from 0.1  $\mu\text{M}$  to 1.5  $\mu\text{M}$ .

25 11. The method of claim 1 wherein the non-nucleic acid polyanion is at a final reaction concentration of from 0.2  $\mu\text{M}$  to 1.0  $\mu\text{M}$ .

12. A method of polynucleotide synthesis, comprising:

combining in a polymerization reaction mixture a thermostable polymerase, a template nucleic acid molecule, appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic acid  
5 polyanion, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity;

heating the polymerization reaction mixture to a temperature at which the template nucleic acid molecule is denatured from a double-stranded molecule to a  
10 single-stranded molecule;

cooling the polymerization reaction mixture to a temperature at which appropriate primers anneal to the single-stranded molecule; and



modifying the temperature of the polymerization reaction mixture to 60°C to 75°C wherein the non-nucleic polyanion substantially ceases to inhibit thermostable polymerase activity.

13. A method of polynucleotide synthesis, comprising:  
combining in a polymerization reaction mixture a thermostable polymerase selected from the group consisting of DNA polymerase, RNA polymerase, reverse transcriptase, and mixtures thereof, a template nucleic acid molecule, appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic acid polyanion, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity;

heating the polymerization reaction mixture to a temperature at which the template nucleic acid molecule is denatured from a double-stranded molecule to a singlestranded molecule;

cooling the polymerization reaction mixture to a temperature at which appropriate primers anneal to the single-stranded molecule; and

modifying the temperature of the polymerization reaction mixture to a temperature at which the non-nucleic polyanion is substantially dissociated from the thermostable polymerise, wherein the thermostable polymerise recognizes and provides polynucleotide synthesis on primer annealed nucleic acid molecule.

14. The method of claim 13 wherein the reverse transcriptase is a derivative, mutant or chimeric complex of the reverse transcriptase.

15. A kit for polynucleotide synthesis on a target nucleic acid, the kit comprising:

a thermostable polymerase reversibly bound to a non-nucleic acid polyanion; and

an appropriate polymerase reaction buffer.

16. The kit of claim 15 wherein the thermostable polymerase is *Thermus aquaticus*.

17. The kit of claim 15 wherein the non-nucleic acid polyanion is dextran sulfate.

18. The kit of claim 15 further comprising at least one nucleotide 5'-triphosphate.



19. The kit of claim 15 further comprising a pair of primers for the target nucleic acid.
20. The kit of claim 15 wherein the non-nucleic acid polyanion has a molecular weight of from 1,500 to 500,000.
- 5 21. The kit of claim 15 wherein the non-nucleic acid polyanion has a molecular of from 4,000 to 15,000.
22. A composition for polynucleotide synthesis comprising:  
a thermostable polymerase;  
a non-nucleic acid polyanion;  
a polymerase reaction buffer having monovalent cations between 35-  
5 60 mM;  
at least one dNTP;  
a template nucleic acid molecule;  
and appropriate template nucleic acid primers.
23. The composition of claim 22 wherein the non-nucleic acid polyanion  
10 has a molecular weight of from 1,500 to 500,000.
24. The composition of claim 22 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.
25. The composition of claim 22 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 10,000.
- 15 26. The composition of claim 22 wherein the non-nucleic acid polyanion is a synthetic organic polysulfate selected from the group poly(anetholsulfonic acid), polyvinyl sulfate, and 15 polystyrene sulfate.
27. The composition of claim 26 wherein the anionic polysulfate is a sulfated oligo- or polysaccharide.
- 20 28. The composition of claim 27 wherein the sulfated oligo- or polysaccharide is a sulfated polymer or copolymer of the sugars selected from the group consisting essentially of glucose, N-acetyl-glucosamine, galactouronic acid, hyalouronic acid, N-acetyl-galactosamine and fucose.
29. The composition of claim 28 wherein the sulfated polymer or  
25 copolymer of the sugar is selected from the group consisting essentially of dextran sulfate, fucoidan, heparin, heparan sulfate, chondroitin polysulfate, keratan polysulfate, xylan polysulfate, and pentosan polysulfate.



30. The composition of claim 22 wherein the non-nucleic acid polyanion is at a concentration of from 0.1  $\mu$ M to 1.5  $\mu$ M.

31. The composition of claim 22 wherein the non-nucleic acid polyanion is at a concentration of from 0.2  $\mu$ M to 1.0  $\mu$ M.

5 32. The composition of claim 22 wherein the thermostable polymerase is selected from the group consisting essentially of DNA polymerase, RNA polymerase, reverse transcriptase, and mixtures thereof.

33. The composition of claim 32 wherein the thermostable polymerase is a DNA polymerase and the DNA polymerase is from a thermophilic Eubacteria or a  
10 Archaeobacteria.

34. The composition of claim 33 wherein the thermostable polymerase is selected from the group consisting essentially of *Thermus aquaticus*, *T. thermophilus*, *T. brockianus*, *T. flavus*, *T. ruber*, *Tbermatoga maritima*, *Thermoplasma acidophilus*, *Pyrococcus furiosus*, *Pyrococcus woessii*, *Pyrococcus spec.*, *Sulfolobus spec.*, and  
5 mixtures thereof.

35. The composition of claim 32 wherein the thermostable polymerase is a reverse transcriptase and wherein the reverse transcriptase is selected from the group consisting essentially of MmLV reverse transcriptase, AMV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase,  
5 and mixtures thereof.

36. The method of claim 12 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.

37. The method of claim 12 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 10,000.

10 38. The method of claim 8 wherein the modifying of the polymerization mixture to a temperature at which the non-nucleic polyanion is substantially dissociated from the thermostable polymerase is from 60°C to 75°C.